NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

TRIETHANOLAMINE

(CAS NO. 102-71-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(DERMAL STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

November 1999

NTP TR 449

NIH Publication No. 00-3365

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

TRIETHANOLAMINE

(CAS NO. 102-71-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(DERMAL STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

November 1999

NTP TR 449

NIH Publication No. 00-3365

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
M.R. Elwell, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
R.L. Melnick, Ph.D.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
G.S. Travlos, D.V.M.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Integrated Laboratory Systems

Battelle Columbus Laboratories
Conducted studies, evaluated pathology findings

P.J. Kurtz, Ph.D., Principal Investigator (2-year studies)
A.C. Peters, D.V.M., Principal Investigator (13-week studies)
M.R. Hejtmancik, Ph.D.
L.E. Mezza, D.V.M., M.S.
R.L. Persing, D.V.M.
J.D. Toft, II, D.V.M., M.S.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, M.S., D.V.M.
B.F. Hamilton, D.V.M., Ph.D.

Dynamac Corporation
Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

Analytical Sciences, Inc.
Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
S.R. Lloyd, M.S.

NTP Pathology Working Group
Evaluated slides, prepared pathology report on rats (26 February 1993)

P.K. Hildebrandt, D.V.M., Chairperson
PATHCO, Inc.
F. Chatani, Ph.D.
National Toxicology Program, Observer
S.L. Eustis, D.V.M., Ph.D.
National Toxicology Program
J.R. Hailey, D.V.M.
National Toxicology Program
B.F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
D. Meuten, D.V.M., Ph.D.
North Carolina State University
C.C. Shackelford, D.V.M., M.S., Ph.D.
National Toxicology Program

Evaluated slides, prepared pathology report on mice (26 February 1993)

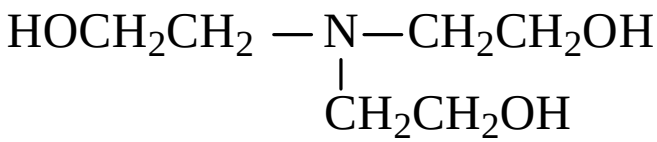
P.K. Hildebrandt, D.V.M., Chairperson
PATHCO, Inc.
S. Botts, M.S., D.V.M.
Experimental Pathology Laboratories, Inc.
R. Cattley, V.M.D., Ph.D.
Chemical Industry Institute of Toxicology
J.R. Hailey, D.V.M.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
K. Takahashi, D.V.M., M.S., Ph.D.
National Toxicology Program

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
G. Gordon, M.A.
L.M. Harper, B.S.
A.M. Macri-Hanson, M.A., M.F.A.
E.S. Rathman, M.S.
W.D. Sharp, B.A., B.S.
S.M. Swift, B.S.

CONTENTS

ABSTRACT



TRIETHANOLAMINE

CAS No. 102-71-6

Chemical Formula: $C_6H_{15}NO_3$ Molecular Weight: 149.19

Synonyms: Nitrilo-2,2′,2′′-triethanol; 2,2′,2′′-nitrilotriethanol; 2,2′,2′′-nitrilotrisethanol; TEA; triaethanolamin-NG; triethanolamin; triethylolamine; tri(hydroxyethyl)amine; 2,2′,2′′-trihydroxytriethylamine; trihydroxytriethylamine; tris(hydroxyethyl)amine; tris(2-hydroxyethyl)amine; triethylolamine; trolamine
Trade Names: Daltogen; Sterolamide; Thiofaco T-35

Triethanolamine is widely used as an ingredient in emulsifiers, thickeners, wetting agents, detergents, and alkalinizing agents in cosmetic products; as a chemical intermediate for anionic and nonionic surfactants and surface active agents in household cleaning agents, textiles, herbicides, pharmaceutical ointments, and other products; as a vulcanization accelerator in the manufacture of rubber; and in many other industrial applications. The National Cancer Institute nominated triethanolamine for study because of its widespread use in cosmetics and other consumer products, its high potential for worker exposure due to its many industrial uses, and its potential for conversion to the carcinogen *N*-nitrosodiethanolamine. Dermal application was chosen as the route of exposure to mimic the principal means of human exposure to triethanolamine and because considerable systemic exposure is achieved with this route. Male and female F344/N rats and B6C3F$_1$ mice received triethanolamine (purity 98% or greater) by dermal application for 13 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, and mouse peripheral blood erythrocytes.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were topically administered 0, 125, 250, 500, or 1,000 mg triethanolamine per kilogram body weight in acetone or 2,000 mg/kg neat triethanolamine, 5 days per week, for 13 weeks. All rats survived to the end of the study. Final mean body weights and weight gains of males and females administered 2,000 mg/kg and the mean body weight gain of females administered 1,000 mg/kg were significantly less than those of the vehicle controls. Clinical observations included irritation, scaliness, and crustiness of the skin at the site of application for males and females. Males also had discoloration, and two males administered 2,000 mg/kg had ulceration at the site of application. Changes in clinical pathology parameters were minor and consistent with inflammation at the site of application.

Kidney weights were generally greater in males and females administered 500, 1,000, or 2,000 mg/kg than in the vehicle controls. Microscopic lesions attributed to triethanolamine administration included acanthosis and inflammation at the site of application,

nephropathy in females, and hypertrophy of the pituitary gland pars intermedia in males and females. These lesions generally occurred with dose-related increases in incidence and severity in males and females.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female mice were topically administered 0, 250, 500, 1,000, or 2,000 mg triethanolamine per kilogram body weight in acetone or 4,000 mg/kg neat triethanolamine, 5 days per week, for 13 weeks. All mice survived to the end of the study. The final mean body weight and weight gain of males in the 250 mg/kg group were less than those of the vehicle controls. Clinical findings were observed only in mice in the 4,000 mg/kg groups and included scaliness, irritation, and discoloration at the site of triethanolamine application for males and females and skin erosion at this site in one male.

The absolute kidney and liver weights of males and females administered 4,000 mg/kg were greater than those of the vehicle controls; relative kidney weights of males administered 1,000 mg/kg or greater and females in all dosed groups were also greater than those of the vehicle controls.

Microscopic examination of the skin of dosed mice indicated acanthosis and inflammation at the site of application. Acanthosis occurred in all dosed groups and in one vehicle control female; the severity increased with increasing dose in males and females. Inflammation was observed in males and females in the 4,000 mg/kg groups and in one female in the 2,000 mg/kg group.

2-YEAR STUDY IN RATS

Based on the presence of acanthosis and inflammation at the site of application at the higher doses in the 13-week study, triethanolamine doses selected for the 2-year study in rats were 32, 63, and 125 mg/kg for males and 63, 125, and 250 mg/kg for females. Groups of 60 male and 60 female rats were topically administered triethanolamine in acetone 5 days per week for 103 weeks. Ten male and ten female rats from each group were evaluated at 15 months for organ weights and histopathology.

Survival, Body Weights, Clinical Findings, and Organ Weights

The survival rate of females in the 250 mg/kg group was slightly less than that of the vehicle controls. The mean body weight of females administered 250 mg/kg ranged from 9% to 12% less than that of the vehicle controls between weeks 73 and 93. Male and female rats receiving triethanolamine had irritated skin at the site of application; in dosed females, the site of application also had a crusty appearance. The number of animals in which these findings were observed increased with increasing dose. At the 15-month interim evaluation, the absolute left and right kidney weights and relative right kidney weight of females administered 250 mg/kg were significantly greater than those of the vehicle controls.

Pathology Findings

The incidence of acanthosis at the site of application in males administered 125 mg/kg and the incidences of acanthosis, inflammation, and ulceration in dosed females were greater than in the vehicle controls at the 15-month interim evaluation and at the end of the 2-year study. Males in the 125 mg/kg group also had greater incidences of inflammation and ulceration than the vehicle controls, and females receiving 125 or 250 mg/kg had greater incidences of epidermal erosion than the vehicle controls at 2 years. There were no skin neoplasms at or away from the site of application that were considered related to treatment with triethanolamine.

At the end of the study, renal tubule adenomas were observed in seven dosed males and in one vehicle control female and one female in the 63 mg/kg group. One male in the 125 mg/kg group and one female in the 250 mg/kg group had renal tubule hyperplasia. Extended (step-section) evaluation of the kidneys of all male rats revealed additional renal tubule adenomas in one vehicle control male, one male in the 32 mg/kg group, two males in the 63 mg/kg group, and three males in the 125 mg/kg group (including one male from the 15-month interim evaluation). An oncocytoma was also identified in one male in the 32 mg/kg group. Hyperplasia was identified in eight additional vehicle control males and in 19 additional dosed males. The total incidences (combined standard and extended evaluations) of renal tubule adenoma in dosed male rats were slightly greater than the vehicle

control incidence (vehicle control, 1/50; 32 mg/kg, 2/50; 63 mg/kg, 6/49; 125 mg/kg, 4/50). The total incidence of hyperplasia in dosed and vehicle control males was similar (9/50, 8/50, 7/49, 6/50). The severity of hyperplasia in males in the 32 and 125 mg/kg groups was greater than that in the vehicle controls.

2-YEAR STUDY IN MICE

Based on dose-related inflammation at the site of application in the 13-week study, triethanolamine doses selected for the 2-year study in mice were 200, 630, and 2,000 mg/kg for males and 100, 300, and 1,000 mg/kg for females. Groups of 60 male and 60 female mice were topically administered triethanolamine in acetone 5 days per week for 103 weeks. Ten male and ten female mice from each group were evaluated at 15 months for organ weights and histopathology.

Survival, Body Weights, Clinical Findings, and Organ Weights

Survival rates of all dosed groups of males and females were similar to those of the vehicle controls. The mean body weight of males administered 2,000 mg/kg ranged from 8% to 10% less than that of the vehicle controls from week 69 through the end of the study. Clinical findings included irritation and discoloration of the skin at the site of application for most males in the 2,000 mg/kg group and a few females in the 1,000 mg/kg group; males administered 200 or 630 mg/kg also had skin irritation. At the 15-month interim evaluation, the right kidney weights of male mice that received 630 or 2,000 mg/kg and the left kidney weights of males that received 2,000 mg/kg were significantly greater than those of the vehicle controls.

Pathology Findings

Acanthosis and inflammation of the skin were observed at the site of application in male and female mice at the 15-month interim evaluation and at the end of the 2-year study. In males in the 2,000 mg/kg group, the incidences of both lesions were significantly greater than those in the vehicle controls at both time points; however, the severities of acanthosis and inflammation did not increase with dose. At the end of the study, the incidence of inflammation in females in the 1,000 mg/kg group was significantly greater than that in the vehicle controls. One vehicle control male and two males in each of the 630 and 2,000 mg/kg groups had ulcers at the site of application.

At the 15-month interim evaluation, hepatocellular carcinomas were observed in dosed and vehicle control males and hepatocellular adenomas in dosed and vehicle control males and females; however, the incidences were not dose related. Nonneoplastic lesions observed at 15 months included foci of cellular alteration in a few dosed males and females; eosinophilic foci were also observed in two vehicle control females.

At the end of the 2-year study, females in the 1,000 mg/kg group had significantly greater incidences of hepatocellular adenoma and multiple adenomas and a greater combined incidence of hepatocellular adenoma and carcinoma than the vehicle controls (adenoma: vehicle control, 22/50; 100 mg/kg, 22/50; 300 mg/kg, 24/50; 1,000 mg/kg, 40/50; multiple adenomas: 11/50, 9/50, 13/50, 29/50; combined adenoma and carcinoma: 23/50, 26/50, 28/50, 41/50). Females in the 300 mg/kg group had significantly greater incidences of hepatocellular carcinoma (1/50, 4/50, 7/50, 5/50) and eosinophilic foci (9/50, 10/50, 18/50, 16/50) than the vehicle controls.

Incidences of hepatocellular adenoma and multiple adenomas in males in the 2,000 mg/kg group were significantly greater than those in the vehicle controls (adenoma: vehicle control, 27/50; 200 mg/kg, 27/50; 630 mg/kg, 29/50; 2,000 mg/kg, 37/50; multiple adenomas: 17/50, 18/50, 17/50, 29/50). Three males in the 2,000 mg/kg group had hepatoblastomas, and males in this group also had significantly greater incidences of hepatocellular neoplasms (combined) (adenoma, carcinoma, and hepatoblastoma: 31/50, 34/50, 33/50, 42/50) and eosinophilic foci (10/50, 17/50, 11/50, 23/50) than the vehicle controls.

Male mice had a pattern of nonneoplastic liver lesions along with silver-staining helical organisms within the liver which suggested an infection with *Helicobacter hepaticus*. With polymerase chain reaction-based assays and culture, the presence of an organism compatible with *H. hepaticus* was confirmed. An increased incidence of hepatocellular neoplasms in male mice has been shown to be associated with

H. hepaticus infection when hepatitis is also present. Therefore, interpretation of the increased incidence of hepatocellular neoplasms in mice was confounded.

GENETIC TOXICOLOGY

Triethanolamine was not mutagenic in any of the *in vitro* or *in vivo* short-term tests performed by the NTP. It did not induce mutations in *Salmonella typhimurium*, and no induction of sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells exposed to triethanolamine was noted. These *in vitro* tests were conducted with and without S9 metabolic activation.

Triethanolamine did not induce sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster* exposed by feeding or injection. No increase in the frequency of micronucleated erythrocytes was observed in peripheral blood samples of male and female mice that received dermal applications of triethanolamine for 13 weeks.

CONCLUSIONS

Under the conditions of these dermal studies, there was *equivocal evidence of carcinogenic activity** of triethanolamine in male F344/N rats based on a marginal increase in the incidence of renal tubule cell adenoma. There was *no evidence of carcinogenic activity* in female F344/N rats receiving 63, 125, or 250 mg triethanolamine per kilogram body weight. The study in male and female $B6C3F_1$ mice was considered *inadequate*, because the presence of a *Helicobacter hepaticus* infection complicated interpretation of the relationship between triethanolamine administration and liver neoplasms in these animals.

Dosed rats and mice had varying degrees of acanthosis and inflammation, dosed rats had ulceration, and dosed female rats had epidermal erosion at the site of skin application.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Triethanolamine

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses in acetone by dermal application	0, 32, 63, or 125 mg/kg	0, 63, 125, or 250 mg/kg	0, 200, 630, or 2,000 mg/kg	0, 100, 300, or 1,000 mg/kg
Body weights	Dosed groups similar to vehicle controls	250 mg/kg group slightly less than vehicle controls	2,000 mg/kg group slightly less than vehicle controls	Dosed groups similar to vehicle controls
2-Year survival rates	21/50, 11/50, 18/49, 19/50	25/50, 29/50, 25/50, 18/50	46/50, 40/50, 39/50, 41/50	39/50, 40/50, 38/50, 37/50
Nonneoplastic effects	Skin, site of application: acanthosis (1/50, 1/50, 1/49, 9/50); inflammation (0/50, 2/50, 0/49, 8/50); ulcer (0/50, 0/50, 0/49, 5/50) Kidney: severity of hyperplasia (standard and extended evaluations - 1.7, 2.6, 1.5, 2.5)	Skin, site of application: acanthosis (2/50, 10/50, 30/50, 32/50); inflammation (2/50, 10/50, 30/50, 32/50); ulcer (2/50, 7/50, 22/50, 27/50); epidermal erosion (1/50, 6/50, 16/50, 14/50)	Skin, site of application: acanthosis (2/50, 1/50, 6/50, 11/50); inflammation (2/50, 0/50, 7/50, 11/50) Liver: eosinophilic foci (10/50, 17/50, 11/50, 23/50)	Skin, site of application: acanthosis (0/50, 2/50, 1/50, 3/50); inflammation (0/50, 2/50, 2/50, 5/50) Liver: eosinophilic foci (9/50, 10/50, 18/50, 16/50)
Neoplastic effects	None	None	None	None
Uncertain findings	Kidney: renal tubule adenoma (standard evaluation - 0/50, 1/50, 4/49, 2/50; standard and extended evaluations combined - 1/50, 2/50, 6/49, 4/50)	None	Liver: hepatocellular adenoma (27/50, 27/50, 29/50, 37/50); hepatoblastoma (0/50, 0/50, 0/50, 3/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (31/50, 34/50, 33/50, 42/50) Incidences of liver neoplasms in mice could not be interpreted due to the presence of *Helicobacter hepaticus* infection.	Liver: hepatocellular adenoma (22/50, 22/50, 24/50, 40/50); hepatocellular carcinoma (1/50, 4/50, 7/50, 5/50); hepatocellular adenoma or carcinoma (23/50, 26/50, 28/50, 41/50)
Level of evidence of carcinogenic activity	Equivocal evidence	No evidence	Inadequate study (*H. hepaticus* infection)	Inadequate study (*H. hepaticus* infection)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Triethanolamine (continued)

Genetic toxicology	
Salmonella typhimurium gene mutations:	Negative in strains TA98, TA100, TA1535, and TA1537 with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Sex-linked recessive lethal mutations	
Drosophila melanogaster:	Negative when administered in feed or by injection
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo*:	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- ! to ascertain that all relevant literature data have been adequately cited and interpreted,
- ! to determine if the design and conditions of the NTP studies were appropriate,
- ! to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- ! to judge the significance of the experimental results by scientific criteria, and
- ! to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

November 29, 1994

Arnold L. Brown, M.D., Chairperson
University of Wisconsin Medical School
Madison, WI

Thomas L. Goldsworthy, Ph.D.
Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Meryl H. Karol, Ph.D.*, Principal Reviewer
Department of Environmental Occupational Health
University of Pittsburgh
Pittsburgh, PA

Curtis D. Klaassen, Ph.D.
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Claudia S. Miller, M.D., M.S., Principal Reviewer
University of Texas Health Sciences Center
San Antonio, TX

Janardan K. Reddy, M.D.*
Department of Pathology
Northwestern University Medical School
Chicago, IL

Irma Russo, M.D.
Fox Chase Cancer Center
Philadelphia, PA

Louise Ryan, Ph.D.
Division of Biostatistics
Harvard School of Public Health and
Dana-Farber Cancer Institute
Boston, MA

Robert E. Taylor, M.D., Ph.D.
Department of Pharmacology
Howard University College of Medicine
Washington, DC

Mary Jo Vodicnik, Ph.D.*
Lilly MSG Development Center
Belgium

Jerrold M. Ward, D.V.M., Ph.D., Principal Reviewer
National Cancer Institute
Frederick, MD

---

* Did not attend

October 30, 1998

Gary P. Carlson, Ph.D., Chairperson
School of Health Sciences
Purdue University
West Lafayette, IN

A. John Bailer, Ph.D.
Department of Mathematics and Statistics
Miami University
Oxford, OH

Steven A. Belinsky, Ph.D., Principal Reviewer
Inhalation Toxicology Research Institute
Kirkland Air Force Base
Albuquerque, NM

James S. Bus, Ph.D.
Health and Environmental Sciences
Dow Chemical Company
Midland, MI

Linda A. Chatman, D.V.M.*
Pfizer, Inc.
Groton, CT

John M. Cullen, Ph.D., V.M.D.
Department of Microbiology, Parasitology, and Pathology
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

Susan M. Fischer, Ph.D.*, Principal Reviewer
M.D. Anderson Cancer Center
The University of Texas
Smithville, TX

Thomas L. Goldsworthy, Ph.D.*
Integrated Laboratory Systems
Research Triangle Park, NC

Stephen S. Hecht, Ph.D., Principal Reviewer
University of Minnesota Cancer Centers
Minneapolis, MN

Michele Medinsky, Ph.D.
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Jose Russo, M.D.*
Fox Chase Cancer Center
Philadelphia, PA

---

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

November 29, 1994: The draft Technical Report on the toxicology and carcinogenesis studies of triethanolamine first received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee on 29 November 1994. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of triethanolamine by discussing the uses of the chemical, describing the experimental design, reporting on survival and body weight effects, and commenting on possible chemical-related neoplasms and nonneoplastic lesions in rats and mice. The proposed conclusions were *equivocal evidence of carcinogenic activity* in male F344/N rats and male B6C3F$_1$ mice, *no evidence of carcinogenic activity* in female F344/N rats, and *some evidence of carcinogenic activity* in female B6C3F$_1$ mice.

Dr. Bucher noted that *Helicobacter hepaticus* had first been described in the literature in 1994 by a member of the Subcommittee, Dr. Ward. He said the infection is associated with a chronic active hepatitis in many strains of mice and appears to affect males more than females. It causes a focal necrosis and inflammation progressing to hepatocytomegaly, oval cell hyperplasia, and cholangitis. In male mice this appears to lead to an increase in the incidence of liver neoplasms. Dr. J.R. Hailey, NIEHS, described the histopathologic appearance of livers from infected animals and the stain used to identify the bacteria. He reported that infection with *H. hepaticus* was suspected or confirmed in four other NTP studies in mice and that the impact on study interpretation was being assessed. Dr. G.N. Rao, NIEHS, said the presence of the bacteria has been reported in a number of laboratories and animal production facilities around the country. However, he stated that the NTP management procedure in production colonies of terminating and restarting colonies every 2 to 3 years made the problem self-limiting in NTP laboratories and believed that the colonies have been free of *H. hepaticus* since 1991 or before.

Dr. Ward, a principal reviewer, agreed with the proposed conclusions. He suggested that because of the infection, further information on sources of mice should be made clear in the Technical Report and would help to indicate that the infection is limited to certain suppliers of mice. Dr. Ward thought the rationale for using the dermal route was adequate but said the report should indicate that the skin application study was approximately equivalent to a low-dose oral study because of significant skin absorption. Dr. Bucher said there was not sufficient dose-response information to make quantitative comparison of oral versus skin absorption of the chemical.

Dr. Miller, the second principal reviewer, agreed with the proposed conclusions, although she said more clarification was needed on why the level of evidence for carcinogenicity in female mice was *some evidence* rather than *clear evidence*. Because triethanolamine is used extensively in more than 2,500 cosmetics, she said the chemical may also contact mucous membranes, especially around the eyes and mouth, and suggested consideration be given to oral/mucous membrane testing. Dr. Bucher said that Japanese studies using 1% and 2% drinking water solutions did not give any strong indication of carcinogenicity. Dr. Miller wondered how the doses used would compare with doses humans might encounter, e.g., in a 5% cream applied daily to the face. Dr. Bucher estimated from a personal communication that such a human dose would not differ greatly from the dose in rats.

Dr. Karol, the third principal reviewer, was unable to attend the meeting but had submitted her review, which Dr. L.G. Hart, NIEHS, read into the record. Dr. Karol agreed with the proposed conclusions. She said that justification was needed for selection of acetone as the solvent for the studies. Dr. Karol also said that in view of reports that the chemical has sensitization potential, the skin lesions and "active inflammation" should be discussed in connection with possible contact dermatitis. Dr. Bucher agreed that a case could be made for contact dermatitis being associated, but in looking at the lesions histologically, there was little evidence that the inflammatory process had an allergic component. There were no perivascular lymphoid infiltrates or edematous reactions with eosinophilic infiltrates which might be expected if contact dermatitis were present.

There was further discussion about the possible impact of *H. hepaticus* in female mice and whether or not infection could be a confounder in the etiology of the liver lesions as in male mice. Dr. Bucher said the diagnosis of oval cell hyperplasia or karyomegaly was observed in only one mid-dose female mouse, and although an exhaustive evaluation was not performed, the bacteria were not believed to be a factor in female mice.

Dr. Miller moved that the Technical Report on triethanolamine be accepted with the revisions discussed and with the conclusions as written: for male rats and mice, *equivocal evidence of carcinogenic activity*, for female rats, *no evidence of carcinogenic activity*, and for female mice, *some evidence of carcinogenic activity*. Dr. Russo seconded the motion, which was accepted unanimously with seven votes.

Subsequent Investigations: Subsequent to the 29 November 1994 public review, the NTP carried out an extensive investigation into the extent of evidence of *H. hepaticus* infection in NTP studies as well as the apparent influence of this infection on neoplasm rates in all organs in male and female mice (see Appendix L). B6C3F$_1$ mice from 12 NTP 2-year carcinogenesis studies were found to be infected with *H. hepaticus*. Many of the male mice from nine of these studies had an associated hepatitis, and these nine studies were considered "affected" studies. The incidences of neoplasms (both hepatocellular neoplasms and hemangiosarcoma) of the liver, but not of other organs, were found to be increased in control male mice in the affected studies compared to the incidences in control males from 26 unaffected contemporary studies. Other observations further differentiated control male mice from affected and unaffected studies. H-*ras* codon 61 CAA-to-AAA mutations were less common in liver neoplasms in males from affected studies compared to historical and unaffected study controls. In addition, increases in cell proliferation rates and apoptosis were observed in the livers of male mice with *H. hepaticus*-associated hepatitis. These data support the hypothesis that the increased incidence of liver neoplasms is associated with *H. hepaticus* and that hepatitis may be important in the pathogenesis. Therefore, it was concluded that the interpretation of carcinogenic effects in the liver of B6C3F$_1$ mice may be confounded if *H. hepaticus*-associated hepatitis is present, and that studies in which liver neoplasia was the only effect in male mice with evidence of *H. hepaticus*-associated hepatitis should be considered inadequate for evaluation of carcinogenesis.

This evaluation did not reveal a significant influence of *H. hepaticus* infection on the occurrence of any neoplasm type in female mice. Nonetheless, it was decided that to repeat the study with uninfected mice was the only way to rigorously rule out an effect of *H. hepaticus* on the triethanolamine-induced incidence of hepatocellular neoplasms in female mice. Therefore, in 1998 a second evaluation of triethanolamine was begun using the same study design and same chemical in the same laboratory as the study reported in this Technical Report. This study is being performed with male and female B6C3F$_1$ mice.

October 30, 1998: The proposal to change the level of evidence for the male mouse study from *equivocal evidence of carcinogenic activity* to *inadequate study* was brought before the NTP's Board of Scientific Counselors' Technical Reports Review Subcommittee on 30 October 1998. Dr. Bucher briefly reviewed the study findings and outlined the NTP's position concerning the interpretation of studies in which there was evidence of *H. hepaticus* infection. This position and the lines of evidence which led to its adoption were presented to the Subcommittee at their meeting on 11 and 12 December 1996 by Dr. Hailey.

Dr. Belinsky, a principal reviewer, began his comments with several suggestions concerning the discussion of the kidney neoplasm findings in male rats and then turned to concerns regarding the *H. hepaticus* issue. He said that while he understood the NTP's position, he was uncomfortable concluding that the evidence was sufficient to rule out a possible influence of *H. hepaticus* infection on the liver neoplasm response in female mice. He suggested that the study may not, in fact, have been totally adequate and that the conclusion of *some evidence of carcinogenic activity* in female mice be amended to indicate that these animals were infected with *H. hepaticus*.

Dr. Hecht, the second principal reviewer, also said that he had not fully understood that female mice also

were infected with the organism in studies where male mice were infected and manifested microscopic changes in the liver from the infection. Dr. Hecht suggested that the conclusion for female mice should be reconsidered. Both he and Dr. Belinsky indicated that their proposal to reconsider the conclusion was influenced by the fact that the female mouse study was being repeated. Dr. Hecht also asked that additional information be added to the report concerning the possibility that *N*-nitrosodiethanolamine, a potential contaminant, might have influenced the results.

Dr. Bucher responded that the NTP had had technical difficulty with the assessment of possible *N*-nitrosodiethanolamine contamination in the triethanolamine and diethanolamine stocks used in the NTP studies. He pointed out that the potential carcinogenicity of *N*-nitrosodiethanolamine in mice has not been established, so it was difficult to predict the impact of this contaminant.

Dr. Fischer, the third principal reviewer, was unable to attend the meeting, but Dr. Hart read her written comments. She agreed with the proposal to designate the male mouse study as *inadequate* and questioned whether the doses administered to the female mice were high enough to adequately reveal possible neoplastic effects in the kidney.

The panel then returned to the discussion of the adequacy of the female mouse study. After listening to public comment on this issue, Dr. Carlson called for a motion. Dr. Belinsky moved that the conclusions of *equivocal evidence of carcinogenic activity* in male rats, *no evidence of carcinogenic activity* in female rats, and *inadequate study* in male mice, as proposed by the NTP, be accepted by the panel and that the study in female mice be judged *inadequate*. Dr. Hecht seconded the motion, which was accepted by three yes votes (Drs. Belinsky, Hecht, and Medinsky) to two no votes (Drs. Bailer and Cullen), with one abstention (Dr. Bus).